CHAPTER IV

RESULTS AND DISCUSSION

Oxidative reaction is known to be an important stability problem of many drugs and pharmaceuticals. As it was mentioned earlier, the oxidation reaction may be one way of ranitidine HCl degradation. Thus, the purpose of this research is to describe the possibility of ranitidine HCl oxidation accelerated by light and oxygen that are important factors of oxidation. Three different groups of antioxidants: free radical inhibitors, oxygen scavengers and chelating agents were also studied. The high pressure liquid chromatography (HPLC) technique of Gupta (Gupta, 1988) was appropriately modified and validated for assay of ranitidine HCl in the presence of its degradation products.

1. Kinetics of Ranitidine HCl Degradation

Kinetic principles are always of great importance in stability programs. Because the goal of chemical kinetics is to elucidate reaction mechanisms. The fundamental principles are mostly conveniently described by solution kinetics which are best elucidated. Order and rate of reactions are two important parameters that indicate the kinetics of chemical reaction. The order of reaction determines the shape of the concentration-time profile of drugs or drug products, whereas the rate constant determines by its slope (Connors et al., 1986).

The order of reaction can be determined by several ways. The graphic method is one of the convenient method used to determine the order of reaction (Martin, Swarbrick and Cammarata, 1983). If a plot of concentration of drug remaining in a degradation process versus time is a straight line, the reaction kinetic is said to be zero-order. The reaction kinetic is first-order when a plot of log (concentration) versus time gives a straight line. Whereas the second-order is the result of the straight line of

the plot of 1/ (concentration) versus time. In this study, the graphic method was used to determine the kinetic order of reaction. The plots of ranitidine HCl concentration versus time, log (ranitidine HCl concentration) versus time, and 1/ (ranitidine HCl concentration) versus time of all formulations studied are shown in Appendix II, and their correlation coefficients (r) are presented in Table 3-6. Almost all correlation coefficients of the second-order plots gave the highest values. Thus, the apparent second-order kinetics of ranitidine HCl degradation in the conditions studied could be concluded.

The rate of reaction can be expressed as the decrease in concentration per unit of time of any of the reacting substances. Thus, a slope of the straigth line plot which best fit to the order of that reaction can be used to determine the observed degradation rate constant (k) of the reaction (Connors et al., 1986). All of rate constants from the second-order plots are concluded in Table 7.

2. Effects of Oxygen and Light

The difference in degradation rate constant values indicated the effect of each factor studied. Analysis of covariance was used to compare the differences of degradation rate constants under a null hypothesis that all of the degradation rate constant values were not different from one another against an alternative hypothesis that at least one pair of the degradation rate constant values was not equal. P-value is the probability of the case of the null hypothesis. The five percent significant level is normally used as the priori cut-off for significance. If the p-value was more than 0.05, then the null hypothesis was accepted and the differences in the degradation rate constants were said to be statistically insignificant. If the p-value was less than 0.05, then the null hypothesis was rejected and the alternative hypothesis was accepted. The analysis of covariance were performed by using the Statistical Package for the Social Sciences (SPSS) (Bryman and Cramer, 1997).

Table 3 Correlation coefficient (r) of regression lines of zero, first and second order plots of ranitidine HCl stored in the presence of oxygen and light.

Additive added	Correlation coefficient (r)		
	Zero order	First order	Second order
None	0.9408	0.9652	0.9811
0.5 % Cremophor EL®	0.8870	0.9330	0.9670
0.001 % BHT	0.8995	0.9441	0.9729
0.005 % BHT	0.9085	0.9511	0.9791
0.5 % Tween 20®	0.9285	0.9698	0.9837
0.001 % Alpha tocopherol	0.9175	0.9623	0.9902
0.02 % Alpha tocopherol	0.9136	0.9574	0.9819
0.01 % Sodium bisulfite	0.8711	0.9355	0.9612
0.1 % Sodium bisulfite	0.9081	0.9532	0.9835
0.01 % Ascorbic acid	0.8936	0.9333	0.9636
0.1 % Ascorbic acid	0.9074	0.9499	0.9795
0.01 % EDTA	0.8724	0.9160	0.9481
0.075 % EDTA	0.8715	0.9034	0.9293
0.3 % Citric acid	0.8495	0.8702	0.8892
2.0 % Citric acid	0.8918	0.9204	0.9455

Table 4 Correlation coefficient (r) of regression lines of zero, first and second order plots of ranitidine HCl stored in the presence of oxygen but the absence of light.

Additive added	Correlation coefficient (r)		
	Zero order	First order	Second order
None	0.9346	0.9537	0.9664
0.5 % Cremophor EL®	0.9431	0.9610	0.9675
0.001 % BHT	0.9469	0.9643	0.9777
0.005 % BHT	0.8974	0.9189	0.9389
0.5 % Tween 20 ®	0.9105	0.8314	0.9384
0.001 % Alpha tocopherol	0.9475	0.9540	0.9557
0.02 % Alpha tocopherol	0.9174	0.9334	0.9472
0.01 % Sodium bisulfite	0.8920	0.9156	0.9346
0.1 % Sodium bisulfite	0.9195	0.9532	0.9770
0.01 % Ascorbic acid	0.9149	0.9394	0.9541
0.1 % Ascorbic acid	0.8712	0.9003	0.9240
0.01 % EDTA	0.9188	0.9283	0.9299
0.075 % EDTA	0.8570	0.8754	0.8930
0.3 % Citric acid	0.9517	0.9652	0.9750
2.0 % Citric acid	0.9666	0.9746	0.9775

Table 5 Correlation coefficient (r) of regression lines of zero, first and second order plots of ranitidine HCl stored in the presence of light but the absence of oxygen.

Additive added	Correlation coefficient (r)		
	Zero order	First order	Second order
None	0.9702	0.9809	0.9795
0.5 % Cremophor EL®	0.9068	0.9406	0.9662
0.001 % BHT	0.9580	0.9772	0.9880
0.005 % BHT	0.9656	0.9773	0.9863
0.5 % Tween 20®	0.9585	0.9862	0.9920
0.001 % Alpha tocopherol	0.9417	0.9671	0.9836
0.02 % Alpha tocopherol	0.9100	0.9454	0.9722
0.01 % Sodium bisulfite	0.9386	0.9659	0.9824
0.1 % Sodium bisulfite	0.9738	0.9849	0.9816
0.01 % Ascorbic acid	0.8771	0.8832	0.8869
0.1 % Ascorbic acid	0.8755	0.9027	0.9253
0.01 % EDTA	0.9150	0.9407	0.9607
0.075 % EDTA	0.8903	0.9106	0.9304
0.3 % Citric acid	0.8760	0.8980	0.9185
2.0 % Citric acid	0.9173	0.9339	0.9493

Table 6 Correlation coefficient (r) of regression lines of zero, first and second order plots of ranitidine HCl stored in the absence of oxygen and light.

Additive added	Correlation coefficient (r)		
	Zero order	First order	Second order
None	0.9769	0.9727	0.9783
0.5 % Cremophor EL®	0.9740	0.9821	0.9882
0.001 % BHT	0.9398	0.9512	0.9611
0.005 % BHT	0.9319	0.9478	0.9828
0.5 % Tween 20®	0.9778	0.9838	0.9869
0.001 % Alpha tocopherol	0.9458	0.9604	0.9713
0.02 % Alpha tocopherol	0.9896	0.9949	0.9935
0.01 % Sodium bisulfite	0.9332	0.9490	0.9620
0.1 % Sodium bisulfite	0.9569	0.9711	0.9807
0.01 % Ascorbic acid	0.9722	0.9797	0.9847
0.1 % Ascorbic acid	0.8807	0.9100	0.9349
0.01 % EDTA	0.8530	0.8562	0.8564
0.075 % EDTA	0.9584	0.9730	0.9845
0.3 % Citric acid	0.9672	0.9699	0.9692
2.0 % Citric acid	0.9453	0.9595	0.9709

Table 7 Second-order degradation rate constants of ranitidine HCl stored in the four different storage conditions.

Additive added	Rate constant (x 10^6 ml/(mg.hr))			
	+ Oxygen		- Oxygen	
	+ Light ($k_{+O,+L}$)	- Light ($k_{+O,-L}$)	+ Light ($k_{-O,+L}$)	- Light ($k_{-O,-L}$)
None	8.74	7.01	5.97	4.18
0.5 % Cremophor EL®	9.56	7.81	7.02	5.01
0.001 % BHT	10.70	8.54	7.00	4.82
0.005 % BHT	11.00	8.16	7.16	5.14
0.5 % Tween 20®	13.10	9.33	7.78	4.79
0.001 % Alpha tocopherol	11.90	8.56	6.96	4.85
0.02 % Alpha tocopherol	11.70	8.37	6.61	5.03
0.01 % Sodium bisulfite	7.24	6.84	6.39	4.77
0.1 % Sodium bisulfite	10.60	9.96	8.79	5.52
0.01 % Ascorbic acid	11.70	7.44	5.96	4.25
0.1 % Ascorbic acid	8.11	5.10	4.05	3.57
0.01 % EDTA	9.21	7.18	6.33	4.30
0.075 % EDTA	12.40	9.81	6.53	4.10
0.3 % Citric acid	11.60	7.73	4.97	3.94
2.0 % Citric acid	10.20	7.09	5.39	3.76

Bar charts of degradation rate constants of ranitidine HCl in phosphate buffer in different storage conditions are shown in Figure 12. The rank order of the degradation rate constants in different storage conditions were $k_{-O,-L} < k_{-O,+L} < k_{+O,-L} < k_{+O,+L}$; where k was the degradation rate constant of ranitidine HCl, and the subscripts represented storage conditions: -O was the absence of oxygen, +O was the presence of oxygen, -L was the absence of light, and +L was the presence of light. The rank order indicated the degradation rate acceleration by oxygen and light. Since $k_{+O,-L} - k_{-O,-L}$ and $k_{-O,+L} - k_{-O,-L}$ represented the degradation rate acceleration by oxygen and light, respectively, and $k_{+O,-L} - k_{-O,-L}$ were greater than $k_{-O,+L} - k_{-O,-L}$ in all cases (Table 8), it could be concluded that the degradation rate acceleration by oxygen was greater than the acceleration by light. When the rate acceleration by oxygen and light were combined $((k_{+O,-L} - k_{-O-L}) + (k_{-O,+L} - k_{-O,-L}))$ and compared with $k_{+O,+L} - k_{-O,-L}$, the values were comparable in the solutions containing 0.5 %w/v Cremophor EL®, 0.001 %w/v BHT, 0.01 and 0.075 %w/v EDTA, and when there was no antioxidant or surfactant added. The addition effect of oxygen and light could be concluded if $((k_{+O,-L} - k_{-O-L}) + (k_{-O,+L} - k_{-O,-L}))$ were comparable with $k_{+O,+L} - k_{-O,-L}$. Most of the degradation accelerations did not have additive effect of oxygen and light. The presence of synergistic effect of oxygen and light could be proposed if $((k_{+O,-L} - k_{-O-L}) + (k_{-O,+L} - k_{-O,-L})) < k_{+O,+L} - k_{-O,-L}$. The synergistic effects were observed in the solutions containing 0.005 %w/v BHT, 0.5 %w/v Tween 20®, 0.001 and 0.02 %w/v alpha tocopherol, 0.01 and 0.1 %w/v ascorbic acid, and 0.3 and 2.0 %w/v citric acid. However, $((k_{+O,-L} - k_{-O-L}) + (k_{-O,+L} - k_{-O,-L})) > k_{+O,+L} - k_{-O,-L}$, in the case of ranitidine HCl solutions containing 0.01 and 0.1 %w/v sodium bisulfite.

3. Effect of Antioxidants

Three groups of antioxidants: free radical inhibitors, oxygen scavengers and chelating agents were studied. Analysis of covariance was performed to examine the differences in the values of degradation rate constants. And the p-values was used to indicate the differences with the significant level of 0.05.

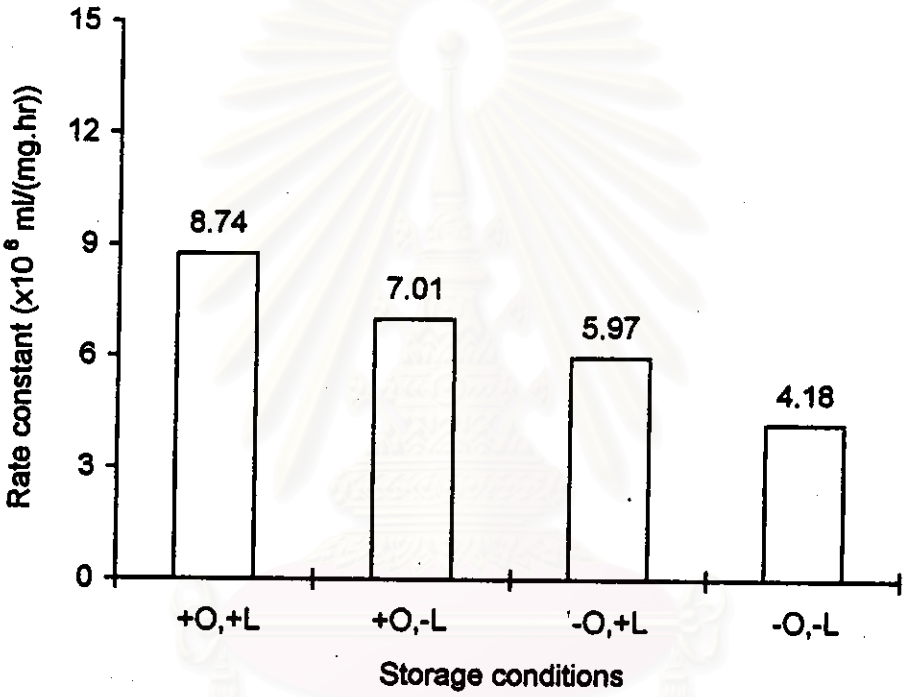


Figure 12 Bar charts of degradation rate constants of ranitidine HCl in phosphate buffer stored in different storage conditions.

Table 8 Additive effect of oxygen and light.

Additive added	$k_{+O,-L} - k_{-O,-L}$ ($\times 10^{-6}$ ml/(mg.hr))	$k_{-O,+L} - k_{-O,-L}$ ($\times 10^{-6}$ ml/(mg.hr))	$(k_{+O,-L} - k_{-O,-L}) + (k_{-O,+L} - k_{-O,-L})$ ($\times 10^{-6}$ ml/(mg.hr))	$k_{+O,+L} - k_{-O,-L}$ ($\times 10^{-6}$ ml/(mg.hr))
None	2.83	1.79	4.62	4.56
0.5 % Cremophor EL®	2.80	2.01	4.81	4.55
0.001 % BHT	3.72	2.18	5.90	5.88
0.005 % BHT	3.02	2.02	5.04	5.86
0.5 % Tween 20®	4.54	2.99	7.53	8.31
0.001 % Alpha tocopherol	3.71	2.11	5.82	7.05
0.02 % Alpha tocopherol	3.34	1.58	4.92	6.67
0.01 % Sodium bisulfite	2.07	1.62	3.69	2.47
0.1 % Sodium bisulfite	4.44	3.27	7.71	5.08
0.01 % Ascorbic acid	3.19	1.71	4.90	7.45
0.1 % Ascorbic acid	1.53	0.48	2.01	4.54
0.01 % EDTA	2.88	2.03	4.91	4.91
0.075 % EDTA	5.71	2.43	8.14	8.30
0.3 % Citric acid	3.97	1.03	5.00	7.66
2.0 % Citric acid	3.33	1.63	4.96	6.44

3.1 Effect of Free Radical Inhibitors

Butylhydroxytoluene (BHT) and alpha tocopherol were the free radical inhibitors studied because they have been widely used. They can also be used in injection preparations. The maximum concentrations used of BHT and alpha tocopherol in parenteral solutions are 0.02 and 0.5 %w/v, respectively (Deluca and Boylan, 1992). Two concentration levels of BHT (about 0.001 and 0.005 %w/v) and alpha tocopherol (about 0.001 and 0.02 %w/v) were studied. Since both compounds are water insoluble, appropriate surfactants were added to improve their solubilities. In the preliminary studies, the surfactants that were found to be appropriate for BHT and alpha tocopherol were 0.5 %w/v Cremophor EL® and 0.5 %w/v Tween 20®, respectively; the surfactant concentrations studied were the maximum concentrations used in the parenteral solutions. The maximum concentrations studied of BHT and alpha tocopherol were the concentrations that they could be soluble in 0.5 %w/v of their surfactants.

3.1.1 Effect of BHT

The effect of Cremophor EL® used for improving the solubility of BHT on the stability of ranitidine HCl is shown in Figure 13. When 0.5 %w/v Cremophor EL® was added, the greater degradation rate constants in all storage conditions were observed. This indicated the acceleration effect of Cremophor EL®. However, the accelerations are not statistically significant since all the p-values, which were used to indicate the differences in rate constants within a storage conditions, are more than 0.05. Since the increments of degradation rate constants in all storage conditions were comparable, the rate acceleration by Cremophor EL® should not involve the oxidative reaction of ranitidine HCl. Kanokwan (1994) found that ranitidine HCl degraded faster in a solution with lower dielectric constant. Therefore, Cremophor EL® that lowered the solution polarity should increase the degradation rate of ranitidine HCl.

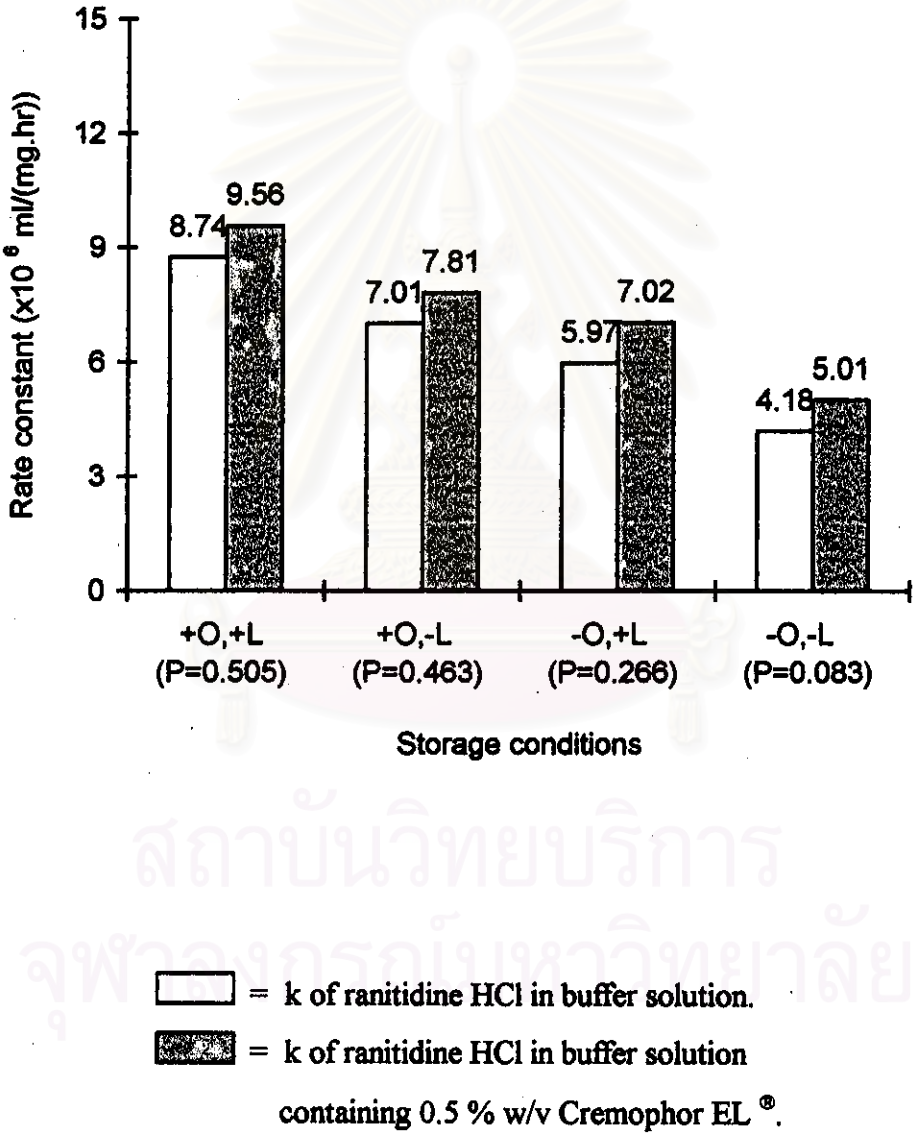


Figure 13 Bar charts presenting the effect of 0.5 %w/v Cremophor EL ®.

When 0.001 and 0.005 %w/v BHT was added to the ranitidine HCl solution containing 0.5 %w/v Cremophor EL®, it seemed that BHT accelerated the degradation rate of ranitidine HCl instead of rate retardation, especially in the cases where oxygen was present (Figure 14). However, the changes in degradation rate constants in each condition are not statistically significant since all the p-values, which were used to indicate the differences in rate constants within a storage condition, are more than 0.05. In general, free radical inhibitors can retard or accelerate the oxidation reaction. When they accelerate the reaction, they are called prooxidants (Chipault, 1962). BHT might act as a prooxidant in the presence of oxygen, especially when light was also present. In the absence of oxygen, BHT concentrations might be too low to work either as a prooxidant or an antioxidant. The two concentrations of BHT studied resulted in similar changes in ranitidine HCl degradation rate. This might be due to the fact that only free BHT molecules in the solutions involved in the degradation of ranitidine HCl. Most of BHT molecules in both concentrations were present in the micelles of Cremophor EL® (CMC = 0.009 %w/v); the free BHT molecules which were saturated in both solutions were comparable. Thus, the concentration effect of BHT could not be observed.

3.1.2 Effect of Alpha Tocopherol

The effect of Tween 20® used for improving the solubility of alpha tocopherol on the stability of ranitidine HCl is shown in Figure 15. The greater degradation rate constants of ranitidine HCl in all storage conditions were observed. However, in the presence of oxygen the accelerations are statistically significant since their p-values, which were used to indicate the differences in rate constants within a storage condition, are less than 0.05. Tween 20® is known to have auto-oxidatively unstable nature. When Tween 20® undergoes auto-oxidation, peroxide formations are observed and the peroxide formations are accelerated by oxygen and light (Ong, Rutherford, and Wich, 1981; Ding, 1993). It was possible that the peroxides formed increased the degradation rate of ranitidine HCl in this study sincethe degradation rate

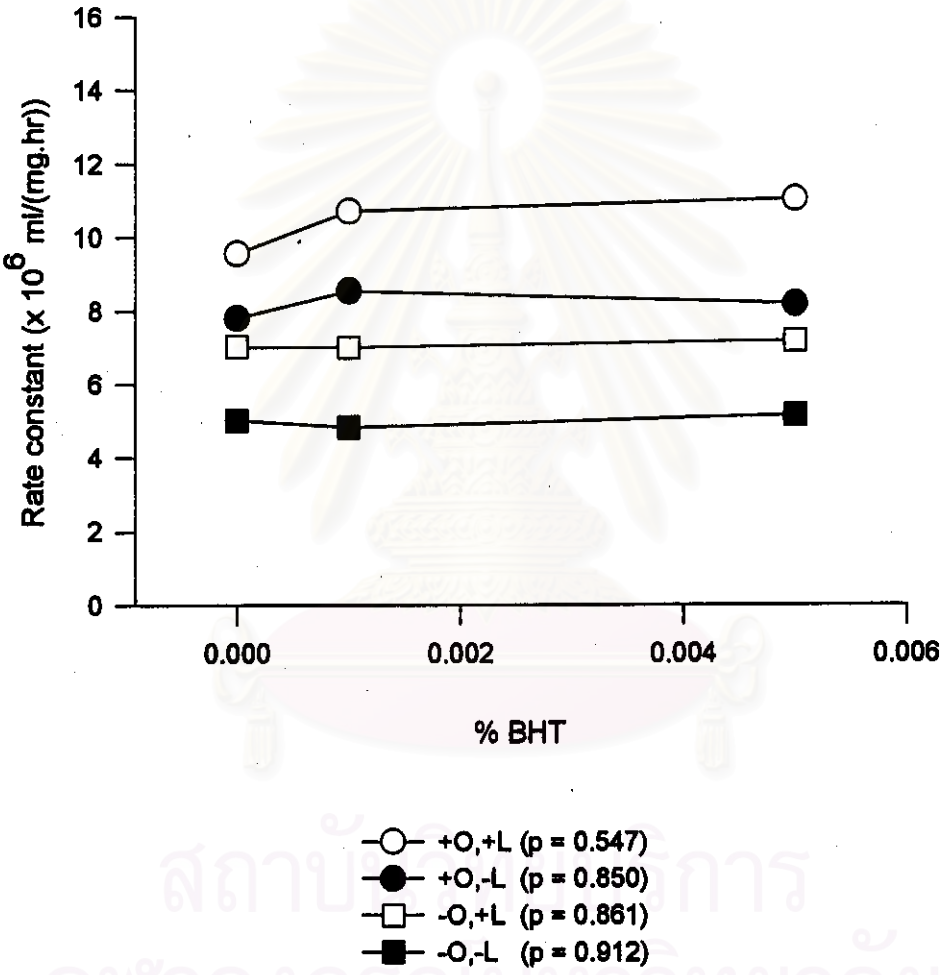


Figure 14 Line graphs presenting the effect of BHT.

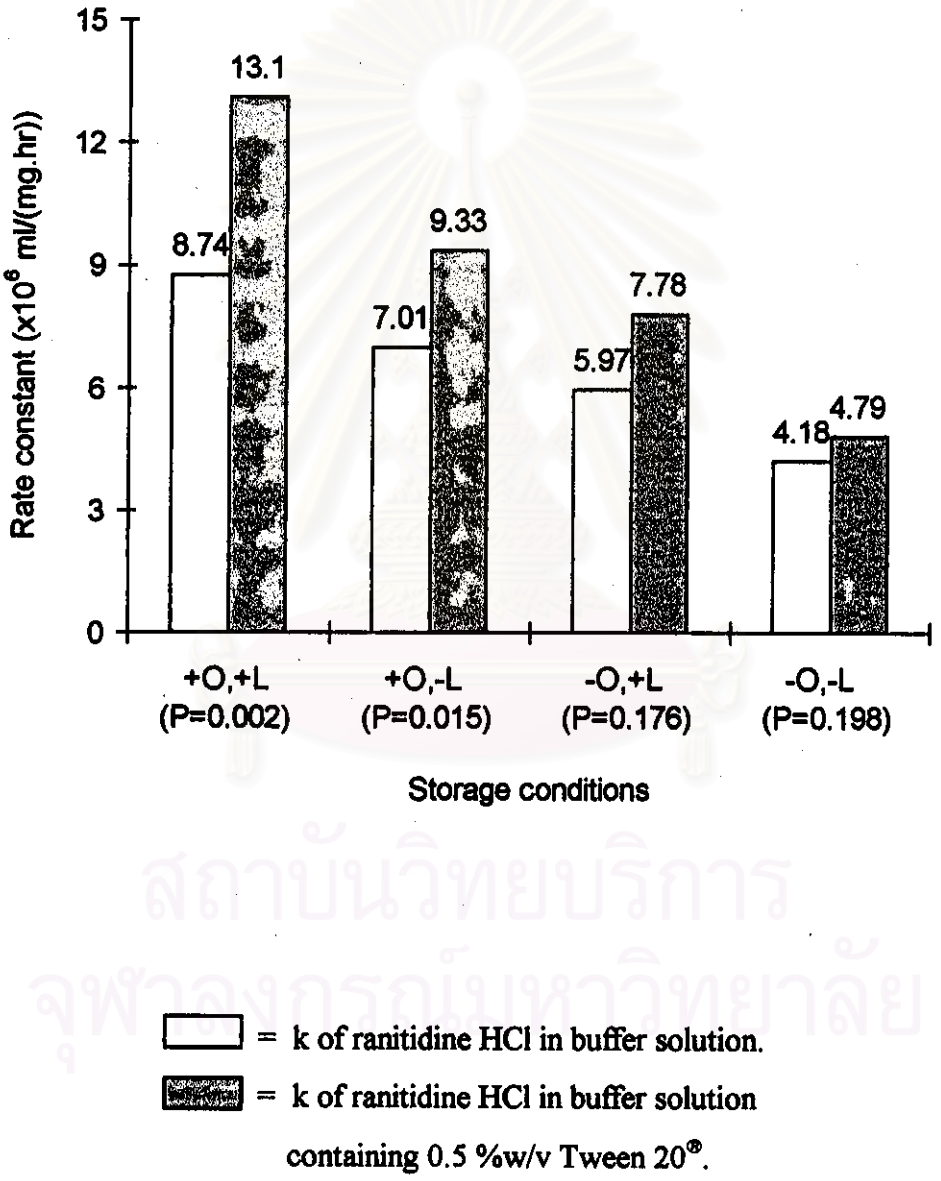


Figure 15 Bar charts presenting the effect of 0.5 %w/v Tween 20®.

increments were greater in the presence of oxygen and / or light. Besides, Tween 20® could accelerate the degradation rate of ranitidine HCl by lowering the dielectric constant of the solution. In the absence of oxygen and light where auto-oxidation of Tween 20® was slight, the acceleration of ranitidine HCl degradation by Tween 20® should be by the lowering of dielectric constant.

When 0.001 %w/v alpha tocopherol was added to the ranitidine HCl solution containing 0.5 %w/v Tween 20®, the degradation rate constants were reduced in all storage conditions except in the condition where oxygen and light were absent (Figure 16). Alpha tocopherol behaves as an effective inhibitor of free radicals that can be explained by the hydrogen donor ability of tocopherol. The donation of a hydrogen atom results in the formation of a chromanoxyl radical which may react with a free radical, for instance, with a peroxyl or another chromanoxyl radical to give either a stable adduct or a nonradical dimer. However, its efficiency is best at low concentration levels and it shows an optimum concentration (Duval and Poelman, 1995). Thus, the auto-oxidation of Tween 20® and ranitidine HCl should be reduced when alpha tocopherol was added, and therefore, the degradation rate constants of ranitidine HCl was decreased. In the +O,+L condition a greater amount of free radicals were formed than that in the +O,-L or -O,+L condition. The greater the amount of free radicals in the solution, the greater the opportunity of alpha tocopherol in inhibition the reaction was. Almost no change was observed when 0.001 %w/v alpha tocopherol was added in ranitidine HCl solution stored in the absence of oxygen and light; this might indicate the little auto-oxidation of Tween 20® and ranitidine HCl.

When 0.02 %w/v alpha tocopherol was added, the same results were observed in all conditions. Duval and Poelman (1995) observed that at a high level of alpha tocopherol, 0.1 % w/v, a decrease in the inhibitory effect of pheomelanin degradation occurrs. They explained that 0.1 %w/v alpha tocopherol shows a tendency to act as a prooxidant. Its prooxidant effect occurs only in aqueous media. At higher concentrations, alpha tocopherol can regenerate its original form by abstracting a hydrogen atom from a stable compound (RH) and induces the formation of a new free

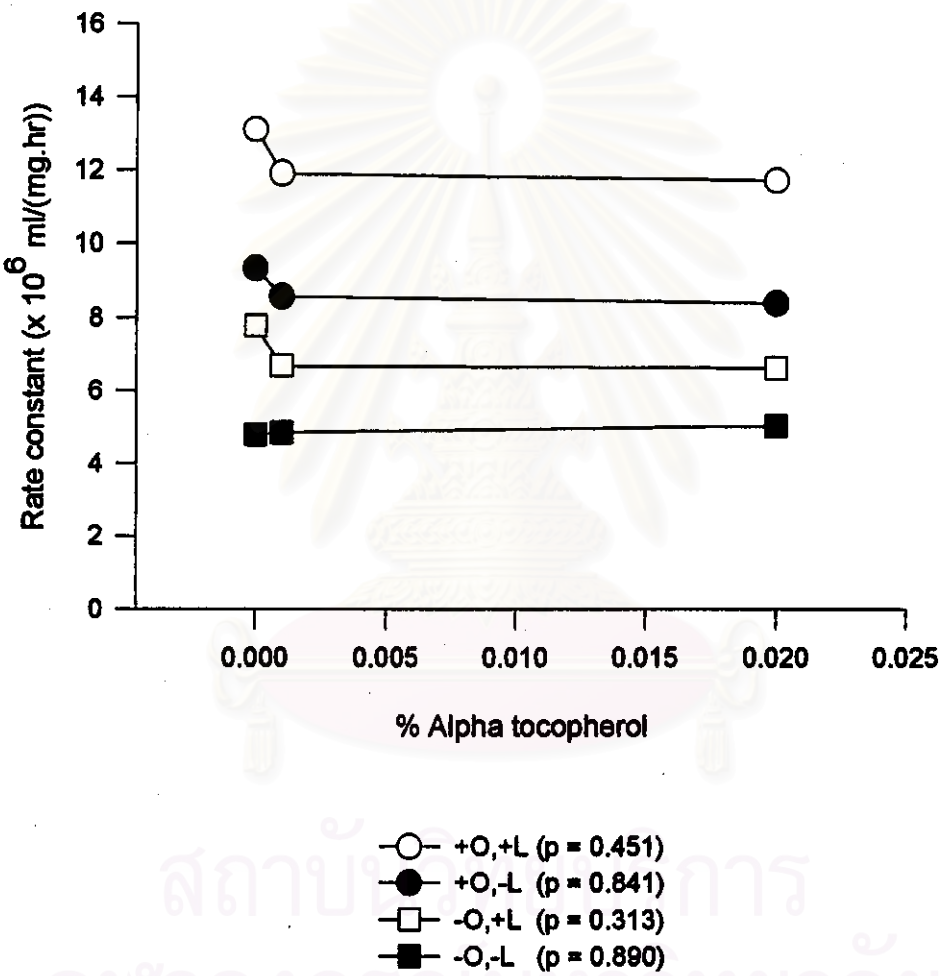


Figure 16 Line graphs presenting the effect of alpha tocopherol.

radical. However, alpha tocopherol did not act as the prooxidant at higher concentration in this study; this might be related to the use of 0.5 %w/v Tween 20® (CMC = 0.001 %w/v) for the solubilization. Almost all of alpha tocopherol molecules might be present in micelles of Tween 20®. Free alpha tocopherol concentration in the solutions containing 0.001 and 0.02 %w/v of alpha tocopherol should be comparable, i.e., its saturated concentration, so the similar results were observed. The changes of degradation rate constants in each condition are not statistically significant since all p-values, which were used to indicate the differences in rate constants within a storage condition, are more than 0.05.

3.2 Effect of Oxygen Scavengers

Sodium bisulfite and ascorbic acid were the oxygen scavengers studied because they have been widely used. The maximum concentration used of either sodium bisulfite or ascorbic acid in parenteral solutions is 0.1 %w/v (Deluca and Boylan, 1992). Two concentration levels of sodium bisulfite (about 0.01 and 0.1 %w/v) and ascorbic acid (about 0.01 and 0.1 %w/v) were studied.

3.2.1 Effect of Sodium Bisulfite

When 0.01 %w/v of sodium bisulfite was added to ranitidine HCl solution, the degradation rate constant of ranitidine HCl was decreased when oxygen was present in the system, but the degradation rate constant was increased when oxygen was absent (Figure 17). The decreasing of degradation rate constant in the +O,+L condition was greater than that in the +O,-L condition. When 0.1 %w/v sodium bisulfite was added, the degradation constants were increased in all storage conditions. The increments of degradation rate constants in the +O,-L and -O,+L conditions were greater than that in the +O,+L condition. The change in degradation rate constants is statistically significant only in the -O,+L condition since the p-value, which was used to indicate the differences in rate constants within a storage condition, is less than 0.05. This result agreed with many reports showing that sodium bisulfite is best effective in optimum lower concentration levels. However, the degradation

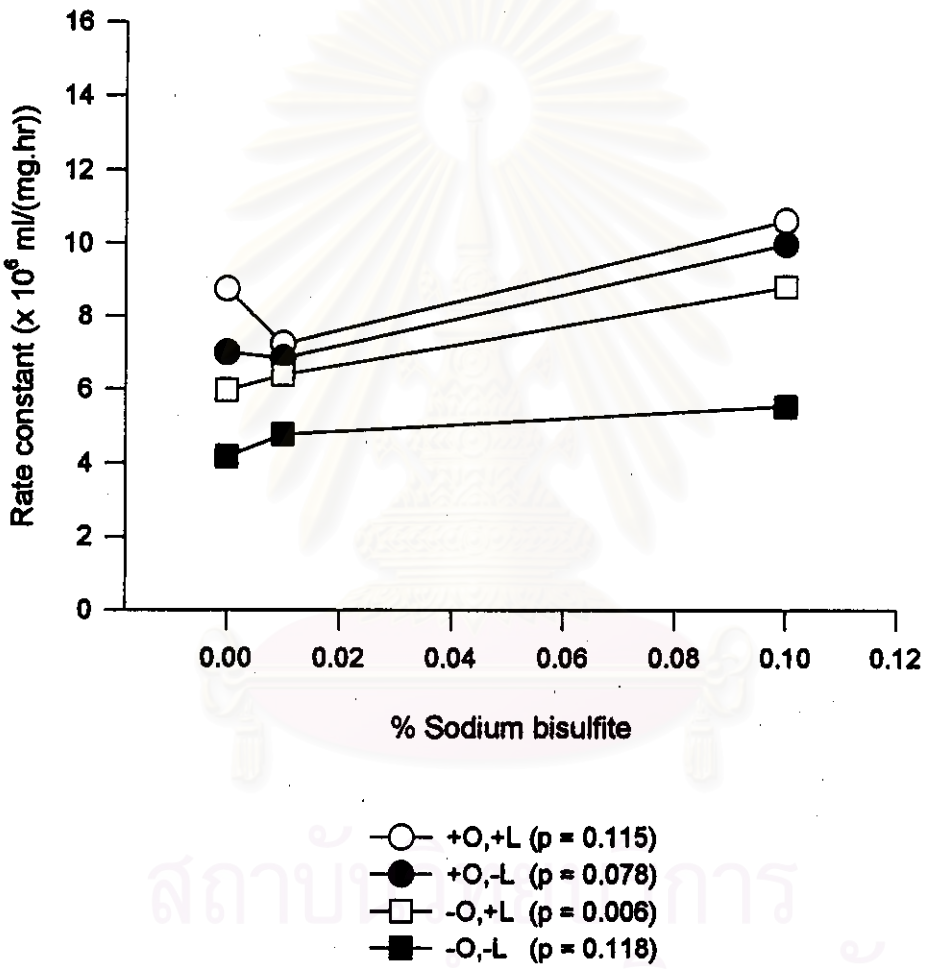


Figure 17 Line graphs presenting the effect of sodium bisulfite.

rate constants decreased only in the presence of oxygen; this might be due to the oxygen scavenger property of sodium bisulfite which could be observed only in the presence of oxygen. Light might involve in its oxygen scavenger property since best oxygen scavenger activity of sodium bisulfite was observed in the presence of oxygen and light. When sodium bisulfite concentration is high, it can accelerate the degradation rate of many drugs and pharmaceuticals. Hussain, Wahner and Triplett (1978) reported that physostigmine degradation increases rapidly when the concentration of sodium bisulfite is increased from 0.001 M to 0.4 M. Fyhr and Brodin (1978) also reported that eprinephrine is more stable in a lower sodium bisulfite concentration. Malkki-Laine and coworkers (1995) found that the best stabilizing effect of sodium bisulfite for salbutamol is at 0.1 %w/v; sodium bisulfite at lower concentration levels is too low to stabilize salbutamol, and at higher concentration levels, it accelerates the decomposition of salbutamol. When the concentration of bisulfite ions is high enough, it can react with alpha-beta unsaturated compounds having a strong electron attracting group on the alpha-position, like mophine, to saturate the double bond and to from a stable sulfonic acid (Yeh and Lach, 1971) and the affinity for these bonds is demonstrated and explained on the basis of a free radical mechanism (Enever et al., 1977). Inactive addition to the alkenes, alkyl halides, and aromatic nitro and carbonyl compounds by sulfite were also reported (Grassby and Hutcuings, 1993). Besides morphine, the addition reaction of sodium bisulfite with tryptophan (Kleinman et al., 1973), fluorouracil (Rork and Pitman, 1975), amitriptyline HCl (Enever et al., 1977), 6-selenoguanosine (Beltagy, Waugh and Repta, 1980), thaimine (Scheiner, Araujo, and Deritter, 1981), sodium nitroprusside (Asker and Canady, 1984), epinephrine (Grubatein and Milano, 1992), and sulbutamol (Malkki-Line et al., 1995) have been also reported.

3.2.2 Effect of Ascorbic Acid

When 0.01 %w/v ascorbic acid was added to ranitidine HCl solution, the degradation rate constants of ranitidine HCl were increased only when oxygen was present, especially when light was also present (Figure 18). In the absence of

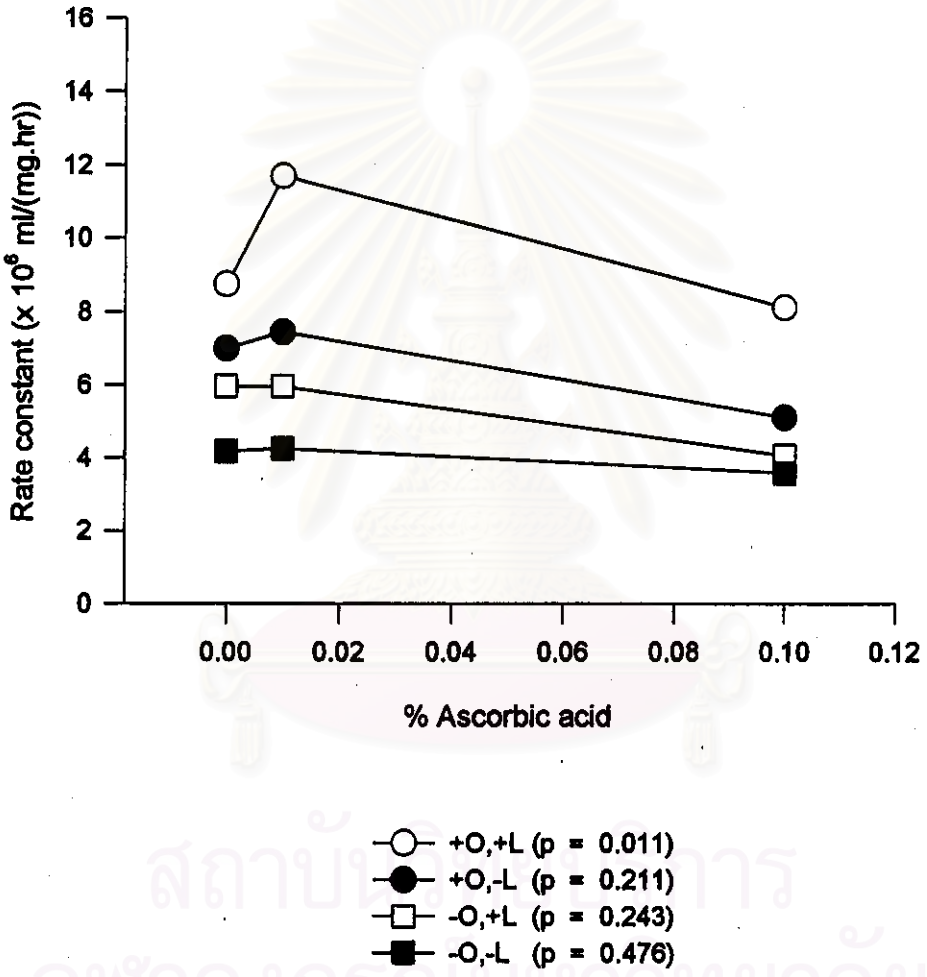


Figure 18 Line graphs presenting the effect of ascorbic acid.

oxygen, the significant acceleration of degradation rate by ascorbic acid was not observed. When the concentration of ascorbic acid was 0.1 %w/v, the degradation rate constants were decreased in all conditions. The decreasings of degradation rate constants in the +O,-L and -O,+L conditions were greater than that in the +O,+L and -O,-L conditions. However, the changes in degradation rate constants in all conditions except for the +O,+L condition are statistically insignificant since the p-value is more than 0.05. In general view, ascorbate ions, which is the soluble form of ascorbic acid, acts mainly as an antioxidant. It is a powerful electron donor and can act as both efficient antioxidant and prooxidant *in vivo* and *in vitro*. Trace amount of metal ions contaminated from a buffer salt is well-known. Ascorbate ion in the presence of oxygen and metal ion can transfer an electron to form reactive oxygen species through metal-catalyzed reaction with oxygen. The formation of superoxide radicals, hydroxyl radicals, and hydrogen peroxide in the metal-catalyzed oxidation of ascorbic acid was reported previously (Li et al., 1993).

$$A + 2Fe^{3+} \longrightarrow \text{Dehydroascorbate} + 2\,Fe^{2+} \quad (27)$$

$$Fe^{2+} + O_2 \longrightarrow O_2^{\bullet-} + Fe^{3+} \quad (28)$$

$$2O_2^{\bullet-} + 2H^{+} \longrightarrow H_2O_2 + O_2 \quad (29)$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{-} + HO^{\bullet} \quad (30)$$

$$HO^{\bullet} + RH \xrightarrow{-O_2} H_2O + ROO^{\bullet} \quad (31)$$

This study also showed the prooxidant effect of ascorbic acid in lower concentration levels in the presence of oxygen, especially when light was also present. Thus, in the absence of oxygen, the absence of prooxidant effect was possible. However, at higher concentration levels, ascorbic acid tended to show the well-known function as an antioxidant. The result was in accordant with the investigation of Wilcox and coworkers (1980) that ascorbic acid at higher concentration level is more effective for apomorphine. Beside the ability to scavenger generated reactive oxygen species, ascorbate could act as a reducing agent and free radical inhibitor, therefore it could lower the degradation rate constants in all conditions.

3.3 Effect of Chelating Agents

EDTA and citric acid were the chelating agents studied because they have been widely used. The maximum concentrations used of EDTA and citric acid in parenteral solutions are 0.075 and 2.0 %w/v, respectively (Deluca and Boylan, 1992). Two concentration levels of EDTA (about 0.01 and 0.075 %w/v) and citric acid (about 0.3 and 2.0 %w/v) were studied.

3.3.1 Effect of EDTA

When 0.01 %w/v EDTA was added to ranitidine HCl solution, the changes in degradation rate constants of ranitidine HCl were slight in all conditions, but when the concentration of EDTA was increased to 0.075 %w/v, the degradation rate constants were increased in the presence of oxygen and / or light (Figure 19). The rate increment was marked when light was also present. However, the changes in degradation rate constants in all conditions are not statistically significant since all the p-values are more than 0.05. In the absence of oxygen, the degradation rate constants were relatively unchanged. In the presence of oxygen, EDTA at higher concentrations doses not simply inhibit the oxidation process by acting as a chelating agent, it can also generate hydroxyl radicals ($HO^{\bullet}$) in the presence of transition metal ions such as iron via a metal-catalyzed Habber-Weiss reaction which can be depicted as follows (Li et al., 1993):

$$Fe^{3+}\text{-EDTA} + O_2^{\bullet -} \longrightarrow Fe^{2+}\text{-EDTA} + O_2 \quad (32)$$

$$2\,O_2^{\bullet -} + 2H^{+} \longrightarrow H_2O_2 + O_2 \quad (33)$$

$$Fe^{2+}\text{-EDTA} + H_2O_2 \longrightarrow Fe^{3+}\text{-EDTA} + HO^{\bullet} + HO^{-} \quad (34)$$

Therefore, EDTA in the presence of oxygen and metal ions might act as a catalyst when its concentration increased. Its catalytic effect could be promoted by light, so the greatest degradation rate constant was observed in the +O,+L condition. Because oxygen involved in the catalytic process, the presence of metal ions showed almost no effect in the absence of oxygen.

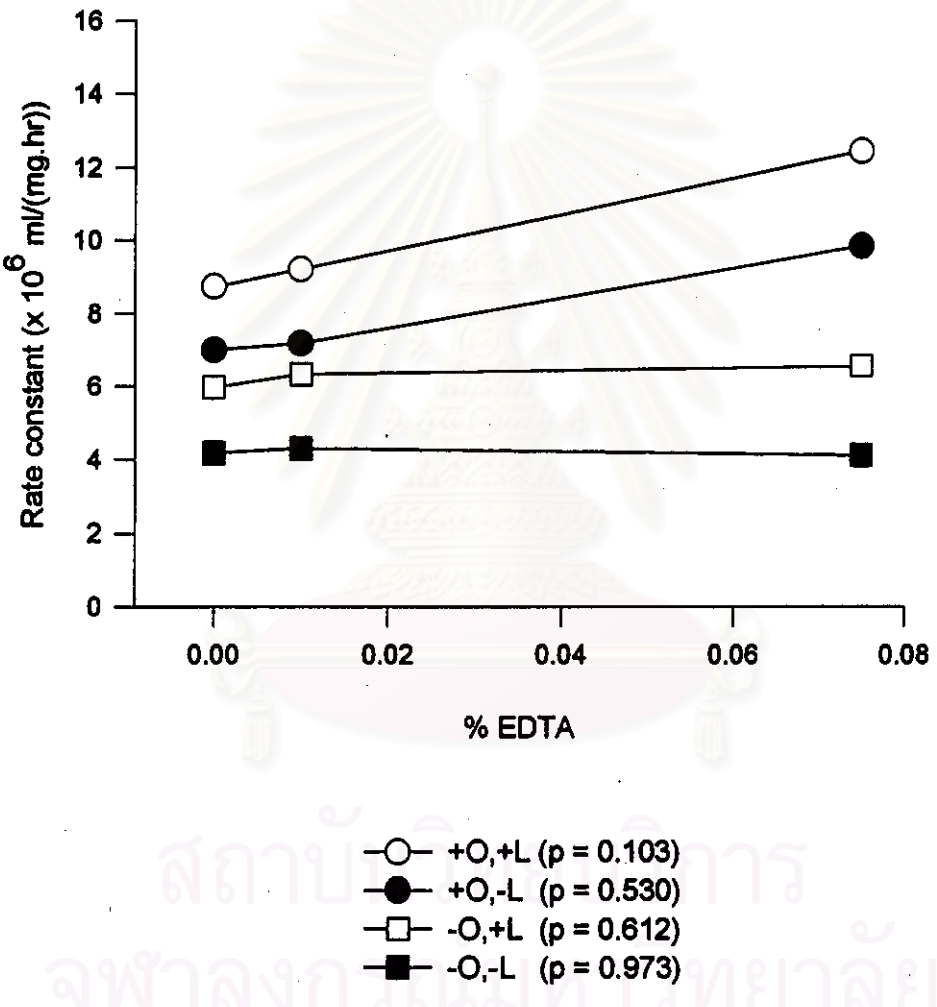


Figure 19 Line graphs presenting the effect of EDTA.

3.3.2 Effect of Citric Acid

When citric acid was added to ranitidine HCl solution, the degradation rate constants of ranitidine HCl were increased in the presence of oxygen, especially when light was also present but the rate constants dropped in the absence of oxygen (Figure 20). The increasing or decreasing of degradation rate constants was greater when light was present. Although the increase in rate constant in the +O,+L condition was appreciable, the changes in degradation rate constants in all conditions are not statistically significant since all the p-values are more than 0.05. Silver and Sundholm (1986) reported that citric acid can interact with codeine to form citrate ester of codeine. In the presence of oxygen, especially when light was also present, the oxidation of ranitidine HCl might produce a compound of which structure favored the interaction with citric acid. In the absence of oxygen, citric acid might work as a chelating agent, especially when light was present.

4. Assay of Ranitidine HCl by HPLC Method

The HPLC method was used for analysis of ranitidine HCl in this stability study because it had high sensitivity and it could isolate the peak of parent compound from the peak(s) of degradation product(s). The ultraviolet spectrum of ranitidine HCl in an aqueous solution and pH 8 phosphate buffer solution are recorded and shown in Figure 21. The spectrum showed two absorption maxima at 229 and 315 nm. The lower wavelength maximum is due mainly to the disubstituted furan chromophore with a contribution from the diaminonitroethene group which has its main absorption at 315 nm (Cholerton et al., 1984). The ultraviolet spectra of ranitidine HCl in pH 8 phosphate buffer containing all kinds of antioxidants were the same. In a preliminary study, the peak of decomposed products showed higher absorptivity at 229 nm with lower resolution, whereas the peak of decomposed products showed lower absorptivity at 315 nm with higher resolution (Figure 22). Procaine HCl used as the internal standard of ranitidine HCl had the maximum absorption at 280 nm. Thus, the wavelength of 290 nm was the wavelength chosen for detection of ranitidine HCl

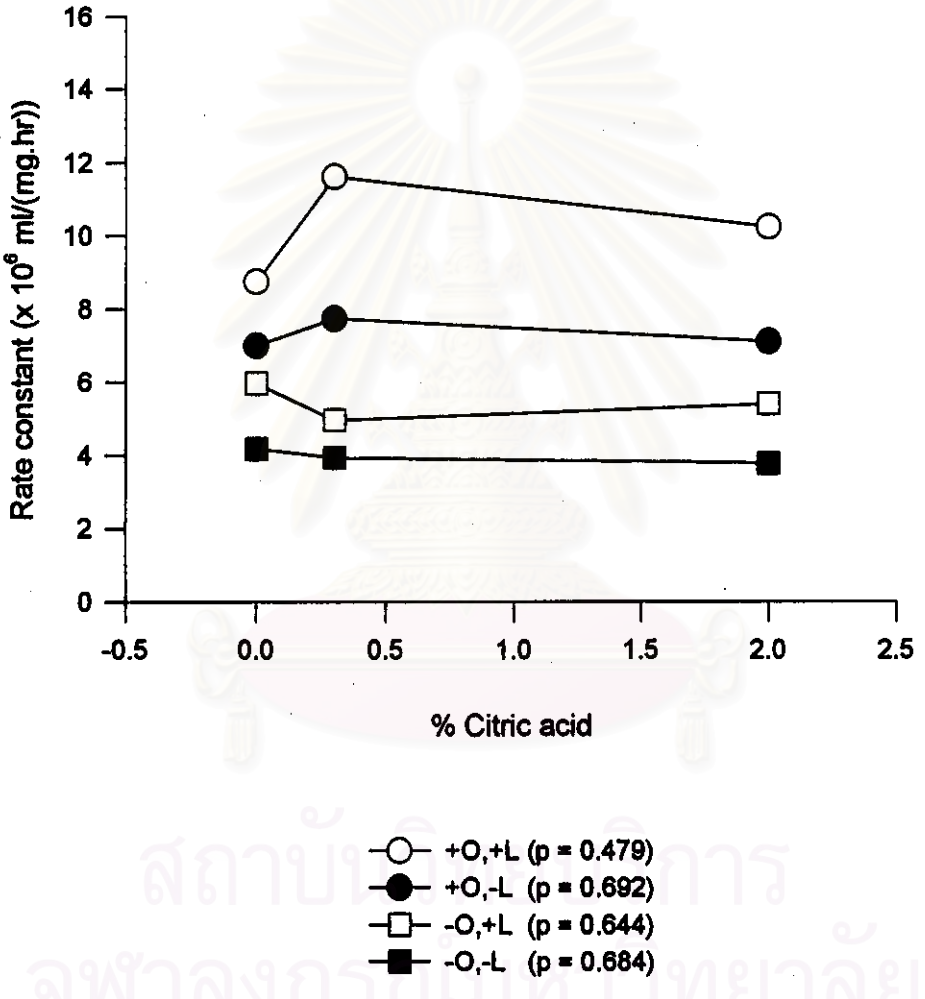


Figure 20 Line graphs presenting the effect of citric acid.

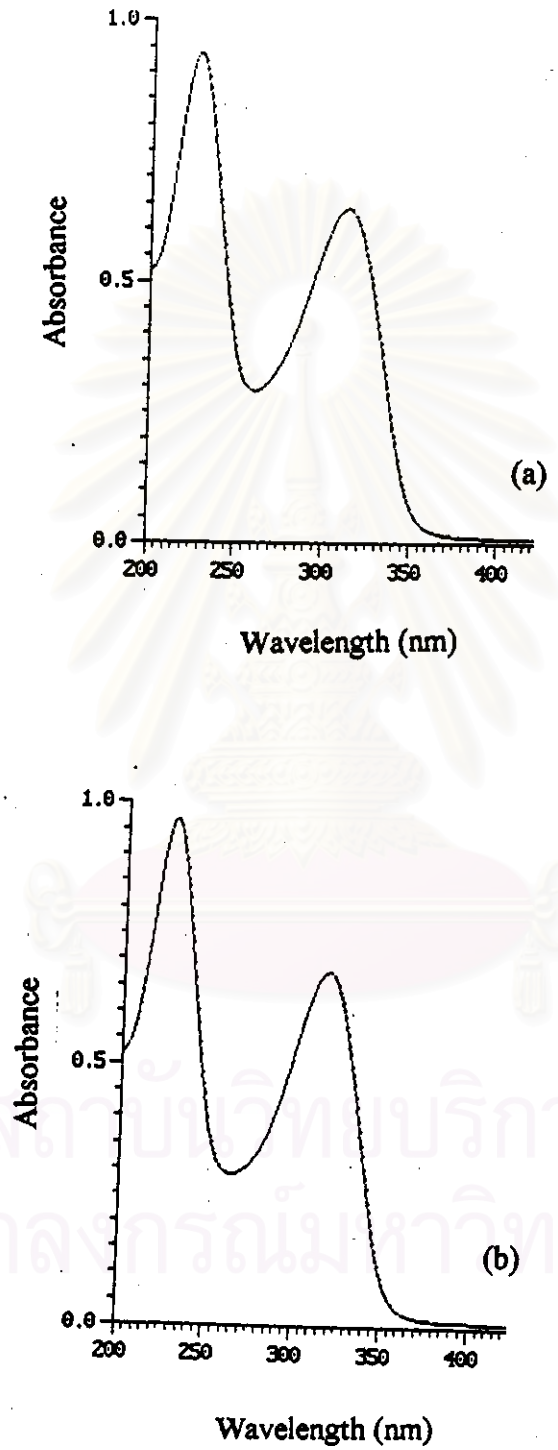
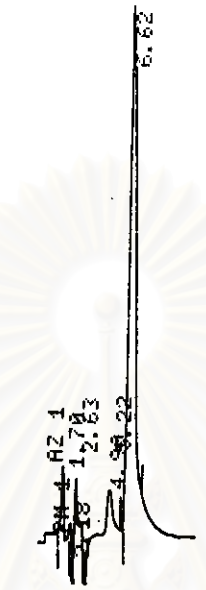


Figure 21 Ultraviolet spectra of ranitidine hydrochloride in an aqueous solution (a) and pH 8 phosphate buffer solution (b).



At 229 nm.

At 315 nm.

Figure 22 Chromatograms of ranitidine HCl at 229 nm and 315 nm.

concentration in the presence of its internal standard, procaine HCl and degradation products.

4.1 HPLC Method validation

4.1.1 Specificity of the Method

Chromatograms of standard solutions (Figure 23) showed that ranitidine HCl and procaine HCl were eluted at 5.00-6.00 min and 8.00-9.00 min, respectively, in all concentrations. Table 9 shows the resolution values of ranitidine HCl and its internal standard, procaine HCl, that were presented as a mean of six replicate injections. All resolution values between ranitidine HCl and procaine HCl peaks were more than 1.0. USP XXIII states that two peaks are completely resoluted if their resolution values are more than 1.0. Thus, the complete separation of ranitidine HCl and procaine HCl peaks could be concluded. Peaks of BHT, alpha tocopherol, sodium bisulfite, ascorbic acid, EDTA, citric acid, Tween 20® and Cremophor EL® did not appear on the chromatograms (Figure 24). It might be due to the lack of absorption of all additives at this wavelength. In conclusion, this method had high specificity for analysis of ranitidine HCl.

4.1.2 Stability Indication

A stability indicating assay is an important methodology to ensure that the capability of the method used in the stability studies is high enough to separate the parent drug from its decomposition products. The chromatograms of decomposed ranitidine HCl in phosphate buffer solution in the presence and absence of additives: BHT, alpha tocopherol, sodium bisulfite, ascorbic acid, EDTA, citric acid, Tween 20® and Cremophor EL®, are shown in Figure 25. All chromatograms were similar. Peaks of decomposition products which had low absorptivity at this wavelength were eluted before that of ranitidine HCl. All peaks of decomposition products were obviously completely resoluted from ranitidine HCl peak whether BHT, alpha tocopherol, sodium bisulfite, ascorbic acid, EDTA, citric acid, Tween 20® and

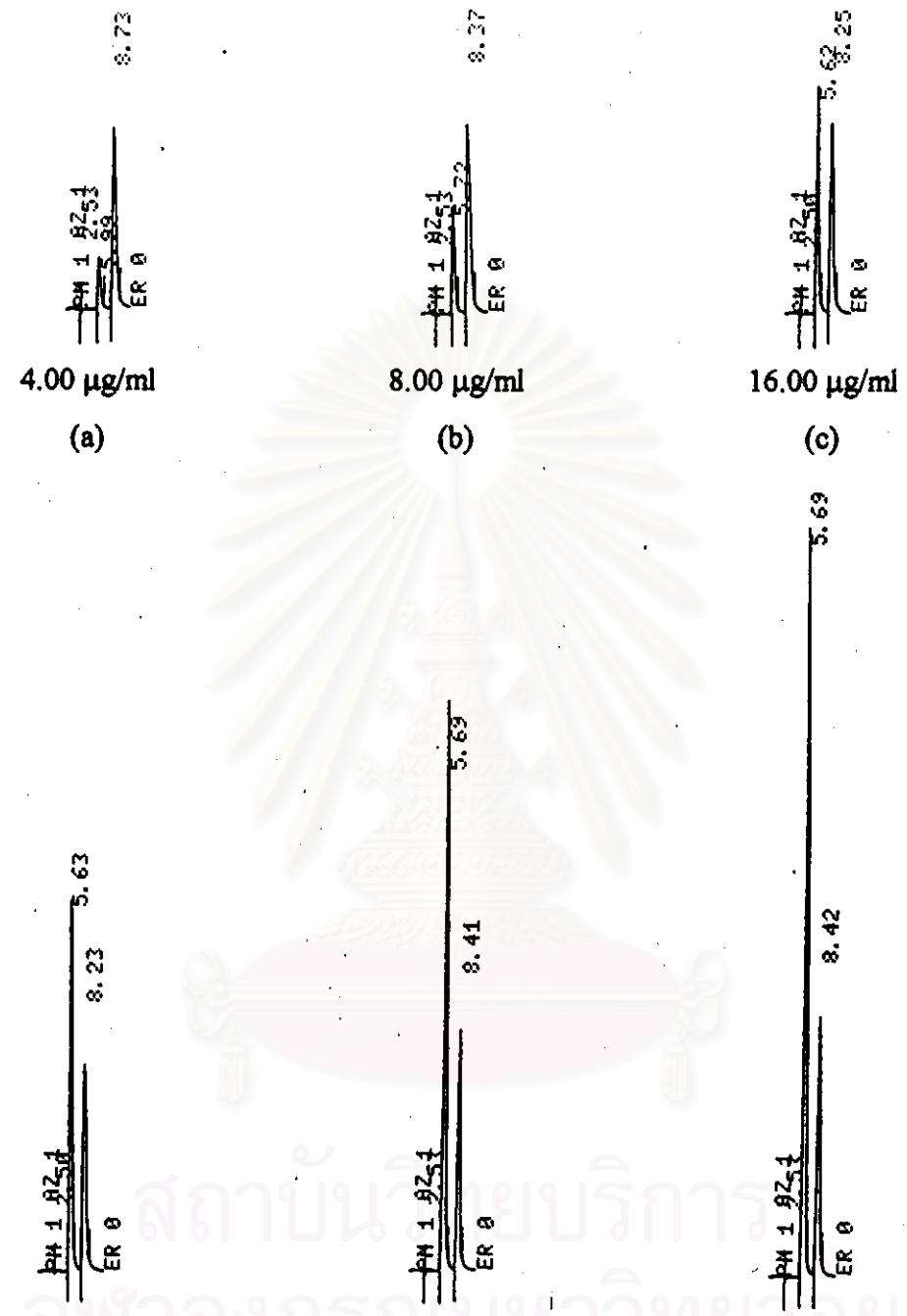


Figure 23 High pressure liquid chromatograms of standard solutions of ranitidine HCl at concentrations of 4.00 µg/ml, (a); 8.00 µg/ml, (b); 16.00 µg/ml, (c); 24.00 µg/ml, (d); 32.00 µg/ml, (e); and 40.00 µg/ml, (f). Retention time of ranitidine HCl and procaine HCl are at 5.00-6.00 and 8.00-9.00 min, respectively.

Table 9 Resolution values of ranitidine HCl and procaine HCl peak.

Concentration of ranitidine HCl (mg/ml)	Resolution values
4.00	2.08
8.00	2.01
16.00	2.10
24.00	2.23
32.00	2.30
40.00	2.25

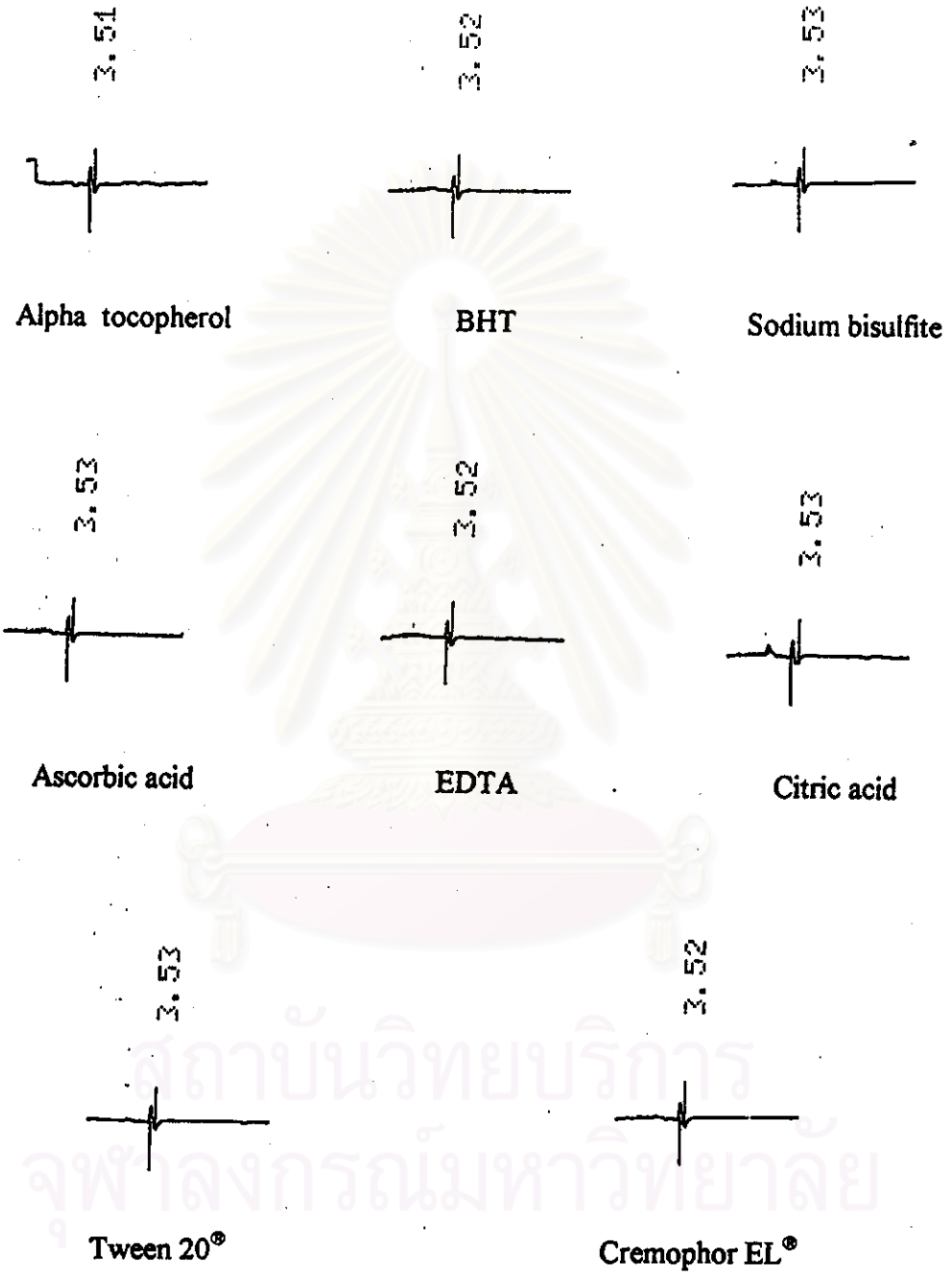
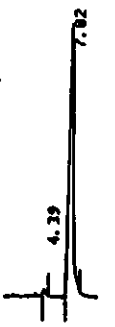


Figure 24 High pressure liquid chromatograms of alpha tocopherol, BHT, sodium bisulfite, ascorbic acid, EDTA, citric acid, Tween 20® and Cremophor EL®.

pH 8 phosphate buffer

Tween 20®



Cremophore EL®

Ascorbic acid

EDTA

Citric acid

Alpha tocopherol

BHT

Sodium bisulfite

Figure 25 High pressure liquid chromatograms of decomposed ranitidine HCl in the presence and absence of additives.

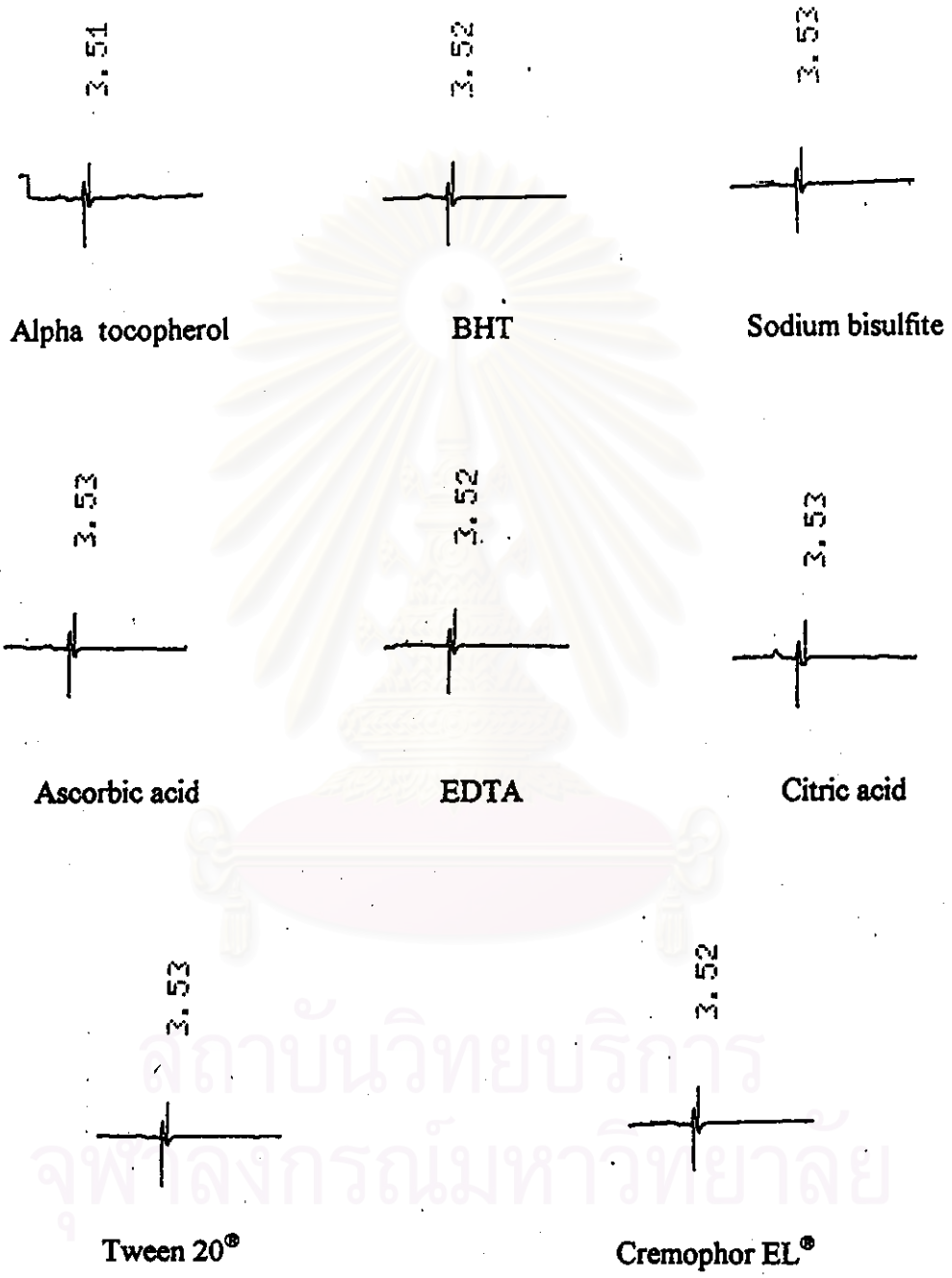


Figure 26 High pressure liquid chromatograms of decomposed alpha tocopherol, BHT, sodium bisulfite, ascorbic acid, EDTA, citric acid, Tween 20® and Cremophor EL®.

Cremophor EL® were added or not. When BHT, alpha tocopherol, sodium bisulfite, ascorbic acid, EDTA, citric acid, Tween 20® and Cremophor EL® were forced to decompose, there was no degradation peak appearing (Figure 26). Therefore, this method could be used for assay of ranitidine HCl in the presence of its degradation products and it was not interfered by the decomposition of other additives used in the formulations.

4.1.3 Linear Correlation

The calibration curve data is shown in Table 10. The plot of ranitidine HCl concentrations versus the peak area ratios of ranitidine HCl and its internal standard (Figure 27) showed the linear correlation in the concentration range studied, 4.00-40.00 μg/ml. The correlation coefficient (r) of this line was 0.9997.

4.1.4 Precision of the Method

The data of within and between run precisions are shown in Table 11 and 12 respectively. The coefficient of variation values of each drug concentration in the within and between run studies were small indicating that this method gave relatively unchanged results for analyzing ranitidine HCl concentration any time.

4.1.5 Accuracy of the Method

The percentages of analytical recovery of each drug concentration are shown in Table 13. All of the mean values were comparable indicating that the percentage of analytical recovery did not depend on drug concentration. The mean percentage of analytical recovery of all drug concentrations which was 99.2 % with a % CV of 1.67 indicated the high accuracy of this method. Therefore, it could be used for analysis of ranitidine HCl in all concentrations studied.

Table 10 Data for calibration curve of ranitidine HCl.

Ranitidine HCl concentrations (μg/ml)	Peak area ratio			
	Set No. 1	Set No. 2	Set No. 3	mean
4.00	0.1746	0.1707	0.1735	0.1729
8.00	0.3910	0.3853	0.3851	0.3873
16.00	0.8083	0.8089	0.8002	0.8058
24.00	1.2391	1.2569	1.2538	1.2499
32.00	1.7453	1.7371	1.7363	1.7395
40.00	2.1423	2.1505	2.1596	2.1508

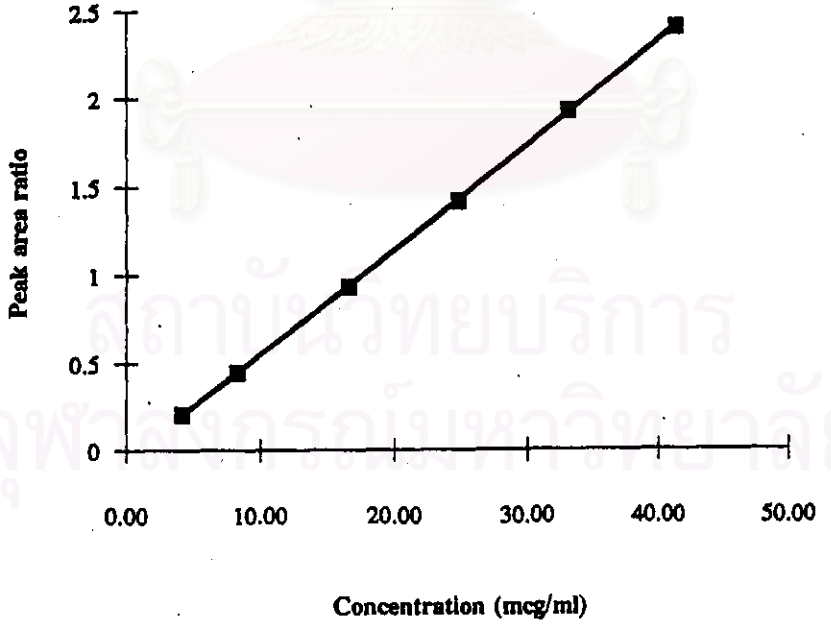


Figure 27 The calibration curve of ranitidine HCl.

Table 11 Data of within run precision.

Ranitidine HCl concentrations (μg/ml)	Peak area ratio				
	Set No. 1	Set No. 2	Set No. 3	Mean (SD)	% CV
4.00	0.1746	0.1707	0.1735	0.1729 (0.0020)	1.16
8.00	0.3910	0.3858	0.3851	0.3873 (0.0032)	0.83
16.00	0.8083	0.8089	0.8002	0.8058 (0.0048)	0.60
24.00	1.2391	1.2569	1.2538	1.2499 (0.0141)	1.13
32.00	1.7453	1.7371	1.7363	1.7395 (0.0050)	0.29
40.00	2.1423	2.1505	2.1596	2.1508 (0.0086)	0.40

Table 12 Data of between run precision.

Ranitidine HCl concentrations (μg/ml)	Peak area ratio				
	Day 1	Day 2	Day 3	Mean (SD)	% CV
4.00	0.1715	0.1773	0.1707	0.1732 (0.0036)	2.08
8.00	0.3755	0.3734	0.3858	0.3782 (0.0066)	1.75
16.00	0.8042	0.7970	0.8089	0.8034 (0.0060)	0.72
24.00	1.2773	1.2858	1.2569	1.2733 (0.0148)	1.17
32.00	1.7561	1.7253	1.7363	1.7392 (0.0156)	0.90
40.00	2.2567	2.1638	2.1596	2.1933 (0.0549)	2.57

Table 13 Percentages of analytical recovery of ranitidine HCl.

Known ranitidine HCl concentration	Concentration calculated from calibration curve	% Analytical recovery
4.00	4.00	101.4
	3.93	98.4
	3.98	99.6
8.00	7.94	99.3
	7.85	98.2
	7.83	98.0
16.00	15.54	97.2
	15.55	97.2
	15.40	96.3
24.00	23.39	97.5
	23.71	98.8
	23.66	98.6
32.00	32.61	101.9
	32.46	101.4
	32.45	101.4
40.00	39.84	99.7
	39.99	99.9
	40.16	100.4
		Mean = 99.2
		SD = 1.66
		% CV = 1.67